UNIVERSITI PUTRA MALAYSIA

ESTABLISHMENT OF NUCLEAR TRANSFORMATION PROTOCOL OF GREEN MICROALGAE Ankistrodesmus convolutus USING ELECTROPORATION

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MASTER OF SCIENCE
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By

VU THI QUYNH CHI

Thesis submitted to the School of Graduate Studies, Universtiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science
February 2013

The thesis was dedicated to

my beloved husband, Dr. Tran Thanh

my parents, Mr. Vu Van Tro and Mrs. Nong Thi Ngoc

my mother-in-law, Mrs. Nguyen Thi Hoan

my brothers and sisters

my nieces and nephews

for their endless love and sacrifice as well as their encouragement which led me to this achievement.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

ESTABLISHMENT OF NUCLEAR TRANSFORMATION PROTOCOL OF GREEN MICROALGAE *ANKISTRODESMUS CONVOLUTUS* USING ELECTROPORATION

By

VU THI QUYNH CHI

February 2013

Chair: Assoc Professor Suhaimi B Napis, PhD

Faculty: Biotechnology and Biomolecular Sciences

Despite the availability of some expression systems such as mammalian cell, yeast, bacteria, insect cell, and fungi, green microalgae have risen as interesting alternative systems for the expression of recombinant proteins. The world of algae is tremendously diverse but the number of species exploited for biotechnological applications is quite limited due to the lack of transformation system and molecular information. As a green microalgae, *Ankistrodesmus convolutus* is a fast growing green microalgae species that contains appreciable amount of carotenoids, polyunsaturated fatty acids and lutein which make this speciesapotential feed in poultry industry. Development of a transformation protocol for this species is essential prior to any of its application for gene manipulation. This study was aimed to establish a nuclear transformation

protocol for *A. convolutus* using electroporation method. The expression vector, pEXP-GUS was created by the modification of pMDC-141 vector. This expressionvectorharbors a β-glucoronidase encoding gene *(gusA)* and ahygromycin phosphotransferase gene (*hpt)* conferringhygromycin resistance driven by double CaMV35S promoter. Minimal inhibitory concentration test of hygromycin showed that non-transformed *A. convolutus* was inhibited at the concentration of 40 mg/L in solid medium after 10 days of inoculation. The parameters of electroporation including cell treatment, electric pulse, concentration of expression vector, concentration of carrier DNA (salmon sperm DNA) and cuvette width were optimized. The results of this study showed that highest transformation efficiency of *A. convolutus* was obtained when usingcells treated with cellulase (2%) and pectinase (0.3%) before transformation and electric pulse of 1800 V. The optimal concentrations of the expression vector and carrier DNA were10 µg/mL and 50 µg/mL, respectively. Electroporation cuvette with 4 mm gap was more efficient than the 2 mm gap cuvette in introducing foreign gene into *A. convolutus*. The highest transformation efficiency was 481 transformants per µg DNA use and the transformation yield was 48 x 10^{-6} cells. A total of 14 transformants were obtained after six rounds of subculturing. The presence of transgenes including *hpt* gene and *gus*A gene were then analyzed by PCR and Southern blot analysis. The resultsrevealed the presence of both *hpt* gene and *gusA* gene in three transformants namely AcG3, AcG4, AcG14. Among these three *A. convolutus* transformants, only one transformants gave hybridization signal when the *Xba*I-digested genomic DNA of PCR-positive transformants was hybridized to a biotin-labelled *gusA* gene-

specific probe. This result implied the integration of *gus*A into the genomes of the transformed cells. The present study was successful in introducing the foreign genes into *A. convolutus* using electroporation. Hence,this success facilitates the expression of foreign genes in this algae species. It is now feasible to exploit *A. convolutus* as an alternative system for the expression of recombinant proteins. Particularly, the prospective of using transgenic *A. convolutus* as oral vaccine or source of recombinant lutein for poultry can be examined.

Abstract tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master di Sains

PENYEDIAAN PROTOKOL TRANSFORMASI NUKLEAR UNTUK MIKROALGAE HIJAU *ANKISTRODESMUS CONVOLUTUS* MENGGUNAKAN KAEDAH ELEKTROPORASI

Oleh

VU THI QUYNH CHI

Februari 2013

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Walaupun terdapat beberapa jenis sistem ekpresi gen seperti sel mamalia, yis, bakteria, sel serangga and kulat, mikroalgae hijau telah dibangunkan sebagai sistem alternatif yang menarik untuk mengekpreskan rekombinan protein. Dunia algae adalah penuh dengan kepelbagaian tetapi bilangan spesies yang dieksploitasikan untuk bioteknologi adalah agak terhad disebabkan kekurangan sistem transformasi dan maklumatbiologi molekul. *Ankistrodesmus convolutus* adalah sejenis spesis mikroalgae hijau yang tumbuh dengan cepat dan mengandungi jumlah karotenoid, asid lemak poli tak tepu dan lutein yang ketara menjadikan spesies ini berpotensi diganakan sebagai makanan didalam industri penternakan haiwanPenyediaan protokol transformasi untuk spesies ini adalah penting untuk sebarang aplikasi bagi manipulasi gen. Kajian ini adalah

bertujuan untuk membangunkan protokol transformasi untuk *A. convolutus* menggunakan kaedah elektroporasi. Vektor ekspresi, pEXP-GUS telah dihasilkan melalui pengubahsuaian vector pMDC 141. Vektor ekspresi ini membawa gen pengekod β-glucoronidase *(gusA)* dan gen hygromycin phosphotransferase (*hpt)* yang membolehkan rintangan terhadap hygromycin dikawalatur oleh promoter CaMV35S yang berganda. Ujian kepekatan perencatan minimal terhadap hygromycin menunjukkan bahawa *A. convolutus* yang tidak ditransformasikan direncat pada kepekatan 40 mg/L dan 20 mg/L dalam media pepejal BBM selepas hari 10 dan dalam media cecair BBM setelah hari 5 masing-masing. Parameter untuk elektroporasi termasuk rawatan sel, kadar arus elektrik, kepekatan vektor pengekspresan, kepekatan DNA pembawa DNA (sperma ikan salmon) dan lebar kuvet telah dioptimumkan. Keputusan untuk kajian ini menunjukkan kecekapan transformasi yang tertinggi telah didapati menggunakan sel yang dirawat dengan selulase (2%) dan pektinase (0.3%) sebelum transformasi dan kadar arus elektrik pada 1800V. Kepekatan optima untuk vektor ekpresi dan pembawa DNA adalah 10 µg/mL dan 50 µg/mL, masing-masing. Kuvet elektroporasi dengan lebar 4 mm adalah lebih sesuai berbanding dengan kuvetlebar 2 mm dalam memperkenalkan gen asing kedalam *A. convolutus*. Kecekapan transformasi yang tertinggi adalah 481 transforman untuk setiap µg DNA yang digunakan dan hasil transformasi adalah 48 x 10^{-6} sel untuk *A. convolutus*. Sejumlah 14 transforman telah diperolehi setelah enam pusingan sub-kulturan. Pengekspresan transgen gen *hpt* dan gen *gus*Atelah dianalisa dengan kaedah PCR dan Southern blot. Keputusan menunjukkan kehadiran untuk kedua-dua gen *hpt* dan gen *gusA* dalam tiga

transforman yang dinamakan AcG3, AcG4, AcG14. Antara tiga transforman *A. convolutus* ini, hanya satu transforman memberi isyarat hibridisasi apabila genomik DNA transforman positif-PCR yang telah dipotong dengan enzim *Xba*I dihibridisasi kepada probe spesifik yang dilabelkan dengan biotin. Keputusan ini menunjukkan integrasi *gus*A kedalam genom pada sel yang ditransformasikan. Kajian ini telah berjaya memperkenalkan gen asing kedalam *A. convolutus* melalui kaedah elektroporasi. Maka,kejayaan ini dapat memudahkan pengekspresan gen asing dalam spesis algae ini. Penemuan ini memungkinkan untuk mengeksploitasikan *A. convolutus* sebagai sistem alternatif untuk pengekspresan protein rekombinan. Secara khususnya, kaedah yang dibangunkanakan berpotensi untukmenjadikan *A. convolutus* transgenik sebagai vaksin oral atau sumber lutein rekombinan untuk penternakan mampu dikaji.

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